Therapy – Intraarticular

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HEPARIN-BINDING IGF-1 PROVIDES SUSTAINED DELIVERY OF IGF-1 TO CARTILAGE IN VIVO

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Purpose: IGF-1 stimulates cartilage growth and repair but is not a practical therapy due to its short half-life in vivo. We modified IGF-1 by adding a heparin-binding domain, creating HB-IGF-1. HB-IGF-1 is retained in bovine cartilage tissue and promotes sustained proteoglycan synthesis compared to IGF-1.
In the present study, we examined the mechanism of HB-IGF-1 retention in cartilage and tested whether HB-IGF-1 could provide sustained in vivo delivery of IGF-1 to rat cartilage after intra-articular injection.
Methods: *Protein Production:* Both HB-IGF-1 and control IGF-1 were expressed with Xpress and 6x-His tags in E. coli and purified by Ni-NTA affinity and reverse-phase chromatography.
Bovine Cartilage with Enzyme Treatment: Cartilage disks (3 mm diam, 0.5 mm thick) from calf femoropatellar grooves were cultured in serum-free medium with 500nM HB-IGF-1 or IGF-1 for 2 days. At Day 2, disks were washed and treated with either no enzyme, chondroitinase ABC (0.4U/mL), or heparitinase (0.036U/mL). At Day 4, half of the chondroitinase-treated disks were treated with heparitinase; all other disks were incubated in enzyme-free medium. On Day 6, IGF remaining in the tissue was detected by Western analysis.
CHO cell binding: Mutant CHO cells lacking heparan sulfate (strain pgsD-677) and wildtype CHO (K1) cells were incubated in serum-free medium with 100nM HB-IGF-1 or IGF-1 for 3 h, washed, and analyzed by Western.
Biacore: Chondroitin sulfate (CS) and heparan sulfate (HS) were biotinylated and bound to a streptavidin-coated Biacore chip. HB-IGF-1 or IGF-1 was flowed across the chip to determine the maximum amount of IGF-1 bound (RUmax).
Intra-articular injection in rat: 10ug HB-IGF-1, 10ug IGF-1, or saline alone was injected into the knee joints of 2-month-old male Sprague-Dawley rats. After one day, joint tissues were harvested and extracted. Portions of extracts with equal total protein were analyzed by Western.
Results: HB-IGF-1 but not IGF-1 remained bound to bovine cartilage explants after 6 days (Fig. 1, No Enzyme). Treatment with chondroitinase ABC greatly decreased binding, while heparitinase had no effect (Fig. 1, C'ase and H'ase). In addition, HB-IGF-1 bound CHO cells lacking HS equally well as wild-type cells, indicating that HS is not required for binding. Biacore analysis showed that HB-IGF-1 bound to both HS and CS at 250-500nM, although HS binding was stronger than CS binding (Fig. 2). Control IGF-1 did not bind any of the GAGs at concentrations up to 1 uM. One day after intra-articular injection, HB-IGF-1 was retained in rat knee articular cartilage, whereas IGF-1 was undetectable (Fig. 3, Articular Cartilage). HB-IGF-1 was detectable despite stronger immunoreactivity of IGF-1 (Fig. 3, Protein Standards). Neither IGF-1 was detected in patellar tendon extracts (Fig. 3, Tendon), consistent with better delivery to the CS-rich cartilage.

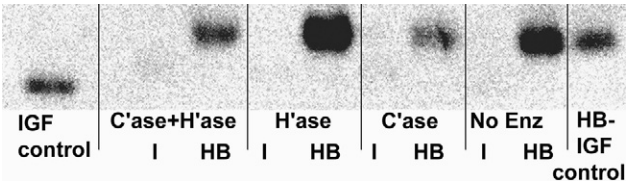

Figure 1

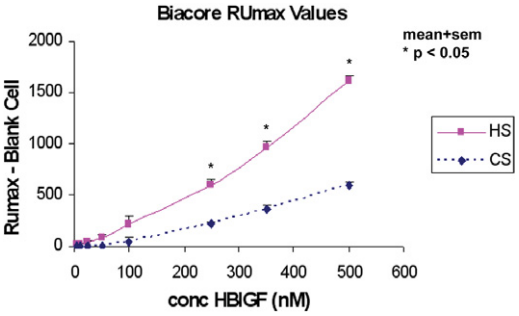

Figure 2

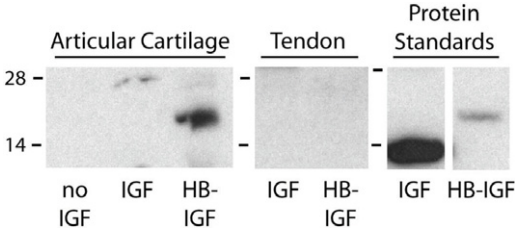

Figure 3

Conclusions: HB-IGF-1 is retained in rat knee cartilage longer than IGF-1 after intra-articular injection. Surprisingly, although HB-IGF-1 binds most strongly to heparan sulfate, the enhanced retention of HB-IGF-1 in cells and cartilage appears primarily due to binding of chondroitin sulfate, which is much more abundant in cartilage than HS. HB-IGF-1 may be a new therapeutic for sustained and relatively specific delivery of IGF-1 to cartilage.

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A SINGLE INTRA-ARTICULAR INJECTION OF INTERLEUKIN 1 RECEPTOR ANTAGONIST IS INSUFFICIENT TO RESTORE GAIT DEFICIENCY RESULTING FROM INTERLEUKIN-1 MEDIATED CARTILAGE DESTRUCTION IN A RODENT MODEL

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Purpose: Interleukin-1 (IL1) plays a pivotal role in osteoarthritis (OA), regulating inflammatory and catabolic processes. IL1 receptor antagonist (IL1Ra) can block IL1-induced inflammation in rheumatoid arthritis, and intra-articular injection of IL1Ra has been evaluated as an OA treatment. IL1-mediated joint inflammation and destruction has been modeled in the rat through intra-articular injection of rat dermal fibroblasts modified to over-express human IL1β. Here, we examine the efficacy of a single intra-articular IL1Ra injection in reversing pain and functional loss in this model.
Methods: Pre-treatment measures of mechanical sensitivity and gait were determined for male Wistar rats (n=14, day=-2). Right knee joints then received a 30 μL injection containing 12,500 rat dermal fibroblasts modified to overexpress human IL1β (day=-1). The following day, rats received either a 30 μL intra-articular injection of IL1Ra (0.65 mg/mL) or saline (n=7, day=0). Gait was quantified on post-treatment day 2 and 6 using high-speed video, and mechanical withdrawal thresholds were measured on post-treatment day 1, 3, and 5.
Results: Following injection of IL1β overexpressing cells, rats locomoted with faster velocities and longer stride lengths on post-treatment day 2 and 6 relative to pre-treatment data ($p<0.001$); however, rats treated with IL1Ra selected slower velocities than rats receiving saline ($p=0.056$). Rats receiving saline demonstrated asymmetric gait on post-treatment day 2 and 6 with a delayed time

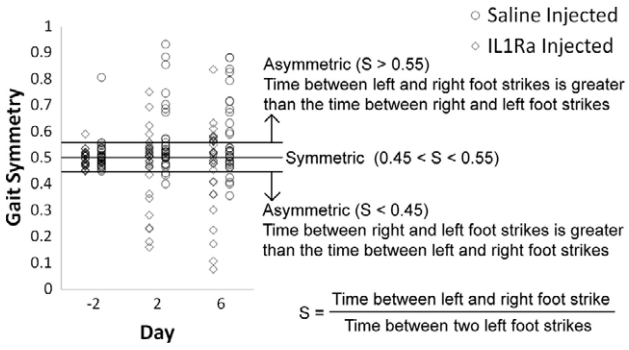

Figure 1

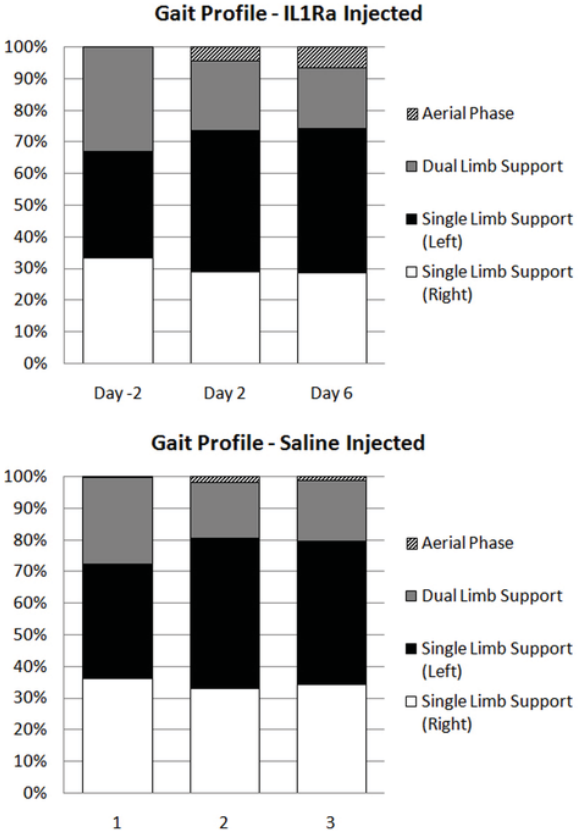

Figure 2

to right foot strike. This may indicate a hesitancy to shift weight from the contralateral limb (left) to the injected limb (right). Asymmetries were also observed in rats treated with IL1Ra; however, asymmetry shifts occurred in both directions (Fig. 1). Both groups demonstrated a strong preference for the contralateral limb on post-treatment day 2 and 6, spending approximately 10% more time on the contralateral limb relative to the injected limb. Furthermore, rats treated with IL1Ra exhibited aerial phases where neither hind limb was in ground contact (Fig. 2). Sensitivity in the injected limb, detected as lower mechanical withdrawal thresholds, increased in both groups with no differences between the groups (Fig. 3).

Conclusions: Rats receiving a unilateral knee injection of hIL1β overexpressing cells locomoted at higher velocities, used asymmetric gait with a preference for the uninjected limb, and exhibited increased mechanical sensitivity. A single treatment with IL1Ra was associated with more symmetric gaits; however, treatment with IL1Ra showed little tendency to modify any other measure at up to 6 days post-treatment. While intra-articular injection of IL1Ra has therapeutic potential for reversing the effects of IL1-mediated

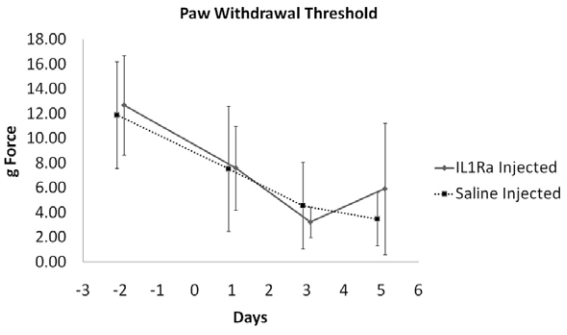

Figure 3

inflammation, these data indicate that a single injection is not sufficient to restore joint function or reduce heightened sensitivity associated with IL1β overexpression. This is likely driven by rapid and efficient IL1Ra clearance from the joint space and motivates studies of sustained intra-articular delivery of IL1Ra.

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ANALGESIC EFFECTS OF INTRA-ARTICULAR BOTULINUM TOXIN TYPE B IN A MURINE MODEL OF CHRONIC DEGENERATIVE ARTHRITIS

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Purpose: New therapies for refractory arthritis pain are needed. Studies have shown that inflammation within joints may cause peripheral and central sensitization of neurons leading to spontaneous joint pain at rest and hyperalgesia with stimulation. Inflammatory mediators induced by neuropeptide release activate peripheral nerve receptors. Given this peripheral sensitization we hypothesize arthritis pain may be treated by intra-articular (IA) neurotoxins. Efficacy of IA botulinum toxin (BoNT) Type A for refractory arthritis pain, and in murine models has recently been reported, prompting interest in screening other botulinum toxins that may prove more effective for arthritis pain. Currently BoNT A and BoNT Type B are the best characterized and most used clinically. BoNT B may produce greater pain relief and may be effective in different types of pain than BoNT A. We hypothesized that BoNT B would reduce chronic arthritic knee pain, testing this in a murine model of chronic degenerative arthritis

Methods: Chronic arthritis was produced in C57Bl6 mice by IA injection of 10 IU Collagenase in 10 μl NS in the left knee peaking at 4 weeks. BoNT B (MYOBLOC) 0.02 IU in 5 μl of NS was given IA in the left knee 3 days before testing. Normal right knee served as internal control. Mice were studied before, after induction of arthritis and after IA BoNT B. Video gait analysis performed using Treadscan™ video gait analysis system hardware and software. In this system lameness was quantified as the standard deviation of stance to stride ratio (STA/STR). Knees were examined for bony swelling indicative of arthritis. Evoked pain behavior was measured by tallying fights + vocalizations/1 min in response to repeated firm palpation of the knee by a single examiner trained for consistency and precision. Gait and strength were observed visually and graded semi-quantitatively as a consensus score among 3 examiners. Strength was measured as ability to grasp and cling. Student's t-test used for statistical comparisons.

Results: Swelling, visual and video gait analysis revealed significant alterations following IA collagenase, indicating induction of arthritis (swelling P= 0.0016, gait P= 0.0081, variability of STA/STR P=0.0128). Evoked pain behaviors increased with arthritis, but were not statistically significant (p=0.3274). Significant improvement in visual gait scores (p=0.0045) and video gait analysis STA/STR variability ($p < 0.0001$) were noted following IA BoNT